CHARGE NUMBER: Project 1904

PROJECT TITLE: Tobacco Physiology and Biochemistry

PERIOD COVERED: September 1-30, 1985

PROJECT LEADER: I.L. Uydess

DATE OF REPORT: October 3, 1985

Objective: To establish the time course and biochemical changes characteristic of tobacco leaves at various stages during senescence.

Status: Two different extractions of greenhouse-grown, Coker 319 green leaf were prepared for use in three biochemical enzyme assays. One set of extractions was made in Pipes buffer, containing KCl, sodium ascorbate, and thiourea, with and without one of two protease inhibitors (Phenyl-Methyl-Sulfonyl-Fluoride, and leupeptin). The second set of extractions was made in Bis-Tris buffer, containing thiourea and KCl, with and without casein and/or leupeptin (a serine and thiol-type protease inhibitor).

Leucine aminopeptidase (LAP), endopeptidase (EP), and carboxypeptidase-A (CP-A) assays were conducted on the aforementiomed green leaf extracts. All positive controls using commercially available preparations of each enzyme worked well. LAP was the only enzyme which showed activity in any of these extracts, as well as in previous extracts.

Protein determinations according to the Bradford method were also made on these extracts. It is not clear at this time whether or not the inclusion of PMSF, casein, and/or leupeptin is necessary.

A method for the determination of chlorophyll in tobacco leaves was transferred from Project 1720 to Project 1904. Frozen green leaf, freeze-dried green leaf, and senesced (yellow) leaf were ground with liquid nitrogen and the chlorophyll extracted with 80% acetone. The absorbancy values were measured at 663 nm and 645 nm and corrected against a background absorbancy at 720 nm. As expected, the amount of chlorophyll was found to be greater in the green leaves, than in the yellow, senesced leaves.

Plans: To continue testing for enzyme activities in tobacco extracts prepared from material grown in the greenhouse or in the field under various conditions.

Other Studies:

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Greenhouse grown, Coker 319 leaf material frozen at -70°C in July, 1985 was powdered and lyophilized to determine the dry weight yield from fresh-frozen leaf at different stages of senescence. This material was subsequently submitted to the Analytical Research Division for the purpose of conducting trial determinations of Ca++, starch, nitrogen, and other targeted tobacco components.

Source: https://www.industrydocuments.ucsf.edu/docs/qfgm0000

Samples supplied by B. Davies were electrophoresed on a 3-27% SDS polyacrylamine gel. This work was done in conjunction with G. Bokelman to characterize proteins present in extractions of an Acetobacter used to study the synthesis of cellulose.

Jan Uyden

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